Catalog Number: 50494

LSM Lymphocyte Separation Medium

Intended Use

For in vitro isolation of lymphocytes from peripheral blood.

Summary of the method

Early methods for isolating leukocytes involved mixing blood with a compound which aggregated the erythrocytes but only slightly affected leukocytes. With centrifugation, erythrocytes pelleted due to their increase density , and leukocytes were collected from the upper part of the tube.

Boyum[3] introduced a more convenient and rapid separation using centrifugation through a Ficoll-sodium metrizoate solution. Diluted blood was layered over the Ficoll-sodium metrizoate solution and centrifuged at a low speed for a short time. Erythrocytes and granulocytes sedimented to the bottom of the tube, and mononuclear cells (lymphocytes) and platelets were collected from the interface between the two phases.

Modifications of the Boyum formulation have been made by numerous workers. "LSM" produced by MP Biomedicals, has a unique formulation using the successful substitution of sodium diatrizoate for the sodium metrizoate.

Principle of the procedure

Defibrinated or heparinized human blood is diluted with physiological saline or balanced salt solution in 1:1 proportion, layered over the separation medium, and centrifuged at a low speed for 30 minutes. During centrifugation, differential migration results in the formation of several cell layers.

The pellet which is formed is comprised mostly of erythrocytes and granulocytes which have migrated through the gradient. Due to their density, lymphocytes and other mononuclear cells (platelets and monocytes) are found at the plasma-LSM interface. Lymphocytes are recovered by aspirating the layer. Further washing removes the platelets, LSM, and plasma.

Reagents

LSM is a sterile filtered solution which contains 6.2g Ficoll and 9.4 g sodium diatrizoate per 100 ml. The density is 1.07700.1-1.0800 g/ml at 20^{o}C.

Precautions

The material is intended for LABORATORY USE ONLY for the in vitro separation of lymphocytes from peripheral blood. **LSM IS NOT INTENDED FOR *IN VIVO* USE.**

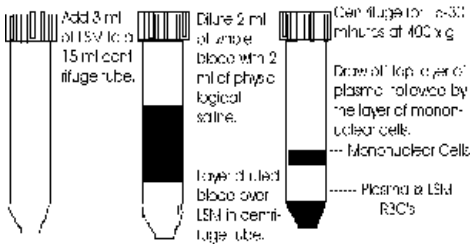
DO NOT USE IF THE MATERIAL IS CLOUDY, HAS A DISTINCT YELLOW COLOR, OR SHOWS ANY SIGNS OF CONTAMINATION.

Stability/storage

Stable until the expiration date listed on the vial. Store at room temperature (18-25^{o}C) in the origin carton. Protect from light.

Instructions for use

The following procedure is one of many variants of the procedure originally described by Boyum. This procedure was developed for use with defibrination or anti-coagulant treated human blood; alterations may be necessary for use with blood from other species or with other tissues.[13-22]



1. Thoroughly mix the LSM by inverting the bottle gently.
2. Aseptically transfer 3 ml of LSM to a 15 ml centrifuge tube.
3. Mix 2 ml of defibrinated, heparinized blood with 2 ml of physiological saline.
4. Carefully layer the diluted blood over 3 ml of LSM (room temperature) in a 15 ml centrifuge tube, creating a sharp blood-LSM interface. DO NOT MIX DILUTED BLOOD INTO THE LSM.
5. Centrifuge the tube at 400 x g at room temperature for 15-30 minutes. Centrifugation should sediment erythrocytes and

polynuclear leukocytes and band mononuclear lymphocytes above LSM (Bands will be Plasma layer -----> Mononuclear cell layer -----> LSM layer -----> RBC pellet) as shown in the diagram above.

6. Aspirate the top layer of clear plasma to within 2-3 mm above the lymphocyte layer.
7. Aspirate the lymphocyte layer plus about half of the LSM layer below it and transfer it to a centrifuge tube. Add an equal volume of buffered balanced salt solution to the lymphocyte layer in the centrifuge tube and centrifuge for 10 minutes at room temperature (18-25^{o}C) at a speed sufficient to sediment the cells without damage. i.e. 160-260 x g . (Washing removes LSM and reduces the percentage of platelets).
8. Wash the cells again with buffered balanced salt solution and resuspend in appropriate medium for your applications.

Store at room temperature (18-25^{o}C) in the original carton. Stable until the expiration date listed on the vial. Protect from light.

Caution : The material is intended for laboratory use only for the *in vitro* separation of lymphocytes from peripheral blood. LSM is not intended for *in vivo* use.

Do not use if the material is cloudy, has a distinct yellow color or shows any signs of contamination.

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